Introduction

There are thousands of chemical reactions taking place in the body of a living organism. The sum of all these chemical reactions is called metabolism. These reactions must take place with a high speed to sustain life. A special group of chemicals responsible for facilitating and speeding up these reactions are called enzymes. Enzymes are mostly protein in nature and coded by genes. They are large group of chemicals which catalyze almost all metabolic reactions in the cell and other parts of the organisms e.g., in digestive tract.

The term enzyme was coined from a Greek word "levened" or "in yeast". First enzyme was discovered by **Payen and Persoz** from germinating barley seeds in 1833 and named it **diastase**. The term enzyme was introduced by **Wilhelm kuhne in 1877**.

Enzymes can be defined as "the **thermolabile biocatalyst**" protein in nature, specific in function and coded by DNA.

They work inside or outside of the cell. The substance on which enzyme acts is called **substrate** which is usually very smaller than enzyme. When enzyme combines with substrate it forms an enzyme-substrate complex. After enzyme substrate reaction product is formed and enzyme itself remains unchanged which can be used again for another substrate. Most enzymes are protein in nature, although a few are catalytic RNA molecules called **ribozymes**, that can catalyze specific substarte in a similar way as proteinaceous enzymes.

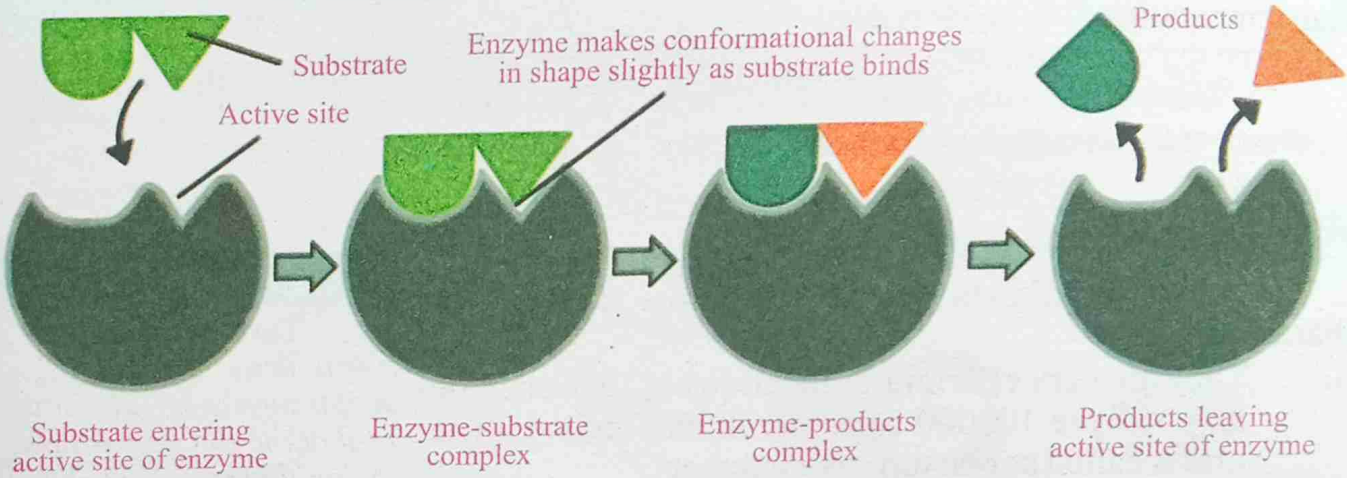


Fig.3.1 Mechanism of Enzyme Action

3.1 Structure of Enzymes

Enzymes are generally globular proteins. The sequence of amino acids specifies the structure of active site which determines the catalytic activity of enzyme. An enzyme may have one or more active sites. Active site of enzyme consists of two parts i.e.,

a. **Binding site** where substrate attaches.

b. **Catalytic site** where catalysis of substrate takes place.

The catalytic site is very small portion comprises of (2 to 12) amino acids.

Do you know?

Ribozyme is found in ribosomes. It controls polypeptide elongation during protein synthesis such as peptidyl transferase.

Chemical Nature of Enzymes:

Most enzymes are proteins, so each has its own specific structure, which is required for its proper functioning. A complete functional enzyme is called holoenzyme.

The **holoenzyme** consists of two parts

a. **Apoenzyme:** It is the proteinaceous part of an enzyme.

b. **Cofactor:** It is non-proteinaceous part of an enzyme.

Apoenzyme + Cofactor = Holoenzyme.

Some enzymes are only composed of protein i.e., no cofactors are attached with them e.g. lipase.

Physical Nature of Enzymes:

Enzymes have relatively high molecular weight e.g., the molecular weight of **peroxidase** is 40,000 Daltons or 40 KDa and **catalase** 250 KDa approximately. Enzymes due to proteinaceous nature may denature in high temperature. The enzymes form colloidal suspension in the cytosol, therefore, at low temperature their activity may decrease or stop. High fever is harmful for the body because enzymes may denature in high temperature.

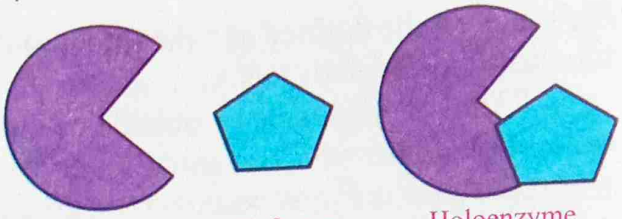


Fig.3.2 Holoenzyme

Activity

If an enzyme breaks three lac moles of substrate in a second. What will be its turnover numbers?

Catalytic Characteristics of Enzymes:

Being catalysts, enzymes show following characteristics.

i) They are very **efficient** in function e.g., one enzyme may catlyze 100000 substrate in one second. (The unit is called as one turnover number).

ii) Enzymes need **specific temperature** for their proper functioning. So drinking cold water during meal is medically wrong.

iii) Enzymes need **specific pH** for their proper functioning.

iv) Enzymes are highly **specific** i.e., one enzyme acts only a specific substrate e.g., amylase acts only on amylose.

v) Enzymes remain constant after the reaction so they can be used again and again.

vi) Enzyme may be studied in living cell ***(in vivo)*** or outside living cell i.e., in glassware ***(in vitro)***.

vii) Most of enzymes need **co-factor** for their functioning.

viii) Enzymes need **aqueous environment** for their functioning, that`s why we feel thirst after taking meal.

Tit bits

Turn over unit

If you turn something over, or if it turn over, it is moved so that the top part is now facing downward or change or reversal of position.

Tit bits

Dalton

A very small unified atomic mass unit (symbol Da) in biology, one hydrogen atom has mass of one Da. The molecular weight of proteins and other macromolecules are usually measured in kilodaltons (KDa).

Three dimensional structure of enzyme:

The enzymes are globular proteins. The specificity of enzymes comes from their unique three dimensional structure. Tertiary structure of a protein or any other macromolecule, play important role in their proper functioning.

The simple protein consists of only one long polypeptide chain e.g., ribonuclease consists of 124 amino acids. The kind of amino acids and the sequence in which they are

arranged determines the three dimensional structure of an enzyme.

Enzyme Cofactors:

Some enzymes do not need additional components to show full activity. However, most of the enzymes require non-protein molecule called cofactors to be bound for activity. Cofactor can be either inorganic metal ions or organic compounds like flavin or haeme. These cofactors serve many purposes e.g., **metal ions** help in making enzyme-substrate complex either by moulding active site or shape of substrate. The **organic substances** may be **co-enzyme** which are released from the enzyme active site during the reaction. They are loosely attached with enzyme. **Prosthetic groups** are tightly bound with enzyme hence the permanent part of enzyme. Most vitamins are co-enzymes or components of co-enzymes. That is why vitamins are needed in our daily life.

3.2 Mechanism of Enzyme Action

Enzymes must bind their substrate before they can catalyze any chemical reaction.

To understand the mechanism of enzyme action two models have been proposed.

Lock and Key Model:

This model was developed by a Germen chemist **Emil Fischer** in 1894.

The specific action of enzyme with a single substrate can be explained using a lock and key analogy. In this analogy the lock is the enzyme and the key is the substrate. Only the correctly sized key that is substrate fits into the key hole which is active site of lock that is enzyme. The same enzyme can be used to catalyze hundreds of same substrates. The enzymes work on this mechanism are called non regulatory enzymes e.g., lipase, amylase etc. This model explains the specificity of enzymes but does not say anything about the change in active site.

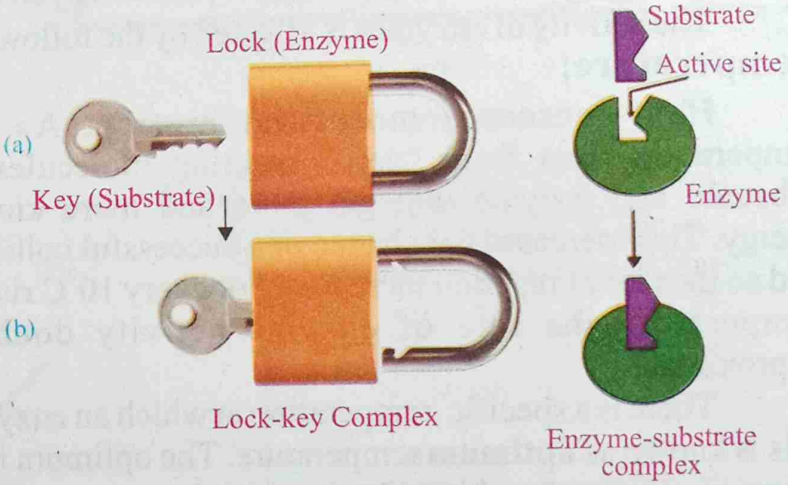


Fig.3.3 Emil Fischer Model

Activity

Study the lock and key enzyme action and induced fit model of enzyme action by animated videos through internet.

Induced-Fit Hypothesis (Model):

In 1958 **Daniel Koshland** suggested a modification to the lock and key model. According to induced-fit model the active site of enzyme is a flexible structure. Enzyme

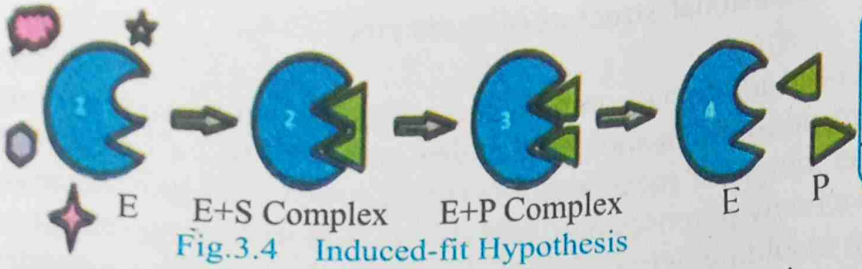


Fig.3.4 Induced-fit Hypothesis

Tit bits

Luciferase is an enzyme in fireflies responsible for light production.

molecules are in an inactive form. To become active, enzymes must undergo slight conformational changes in the structure to accommodate the substrate. A suitable analogy would be that of hand and gloves. The hand corresponds to the substrate and glove as enzyme is shaped by insertion of the hand. Enzymes which follow the induced-fit mechanism are called regulatory or allosteric enzymes e.g hexokinase.

Tit bits

Enzymes are denatured by heat but not by cold thus enzymes stored in below 0°C are able to function after thawing.

3.3 Factors Affecting The Rate of Enzyme Action

The activity of enzymes is affected by the following factors.

Temperature:

Heat increases molecular motion. As the temperature rises from "zero" reacting molecules of substrate and enzyme will get more and more kinetic energy. This increases the chance of a successful collision and so the rate of reaction increases. For every 10°C rise in temperature the rate of enzyme activity doubles approximately.

Do you know?

The food like meat, fruits may turn bad because of the enzyme activity. Therefore, it is advised to keep such food in refrigerator.

There is a specific temperature at which an enzyme catalytic activity is fastest and this is known as **optimum** temperature. The optimum temperature for enzymes found in human is 37°C. After this point the rate of enzyme activity will decrease and at 45-50°C

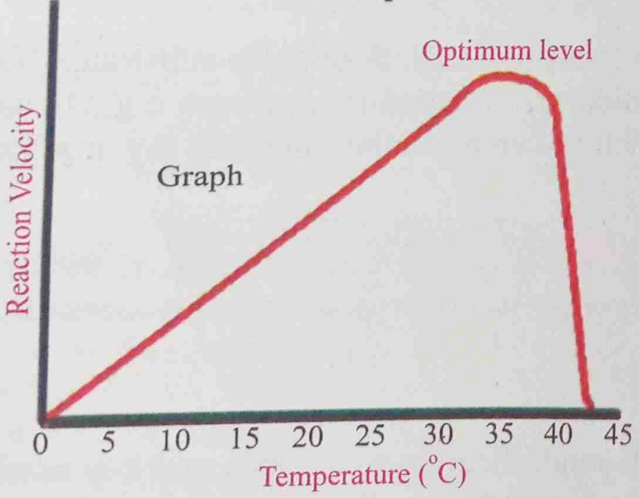


Fig.3.5 Effect of Temperature on enzyme action

Table 3.1 Optimum pH of different enzymes

Enzymes	Optimum pH
Pancreatic Lipase	7.4-7.8
Pepsin	2.0
Trypsin	7.8 - 8.7
Maltase	6.1 - 6.8
Arginase	9.7
Sucrase	4.5

the enzyme activity will be stopped, as enzyme binding site will denature at this temperature. Some bacteria live in hot springs so optimum temperature for their enzymes is more than 37°C. Such enzymes have been used in biological washing powders and detergents. That is why cloth washing need lukewarm water, not too hot.

pH:

Every enzyme needs a specific pH for its proper functioning. The pH at which an enzyme works maximum is called its optimum pH. Some enzymes work best in acidic medium e.g., pepsin, some in neutral medium e.g., amylase and other in alkaline medium e.g., lipase.

However, most of enzymes in our body work in the range of pH 6-8. Some enzymes may work on both acidic and alkaline media e.g., papain enzyme in green papaya.

Change in pH alters the ionic charge of acidic and basidic groups as a result ionic bonding is disrupted. This ionic bonding is needed to maintain the specific shape of enzyme. Thus the change in pH may change the shape of enzyme as well as denature active site.

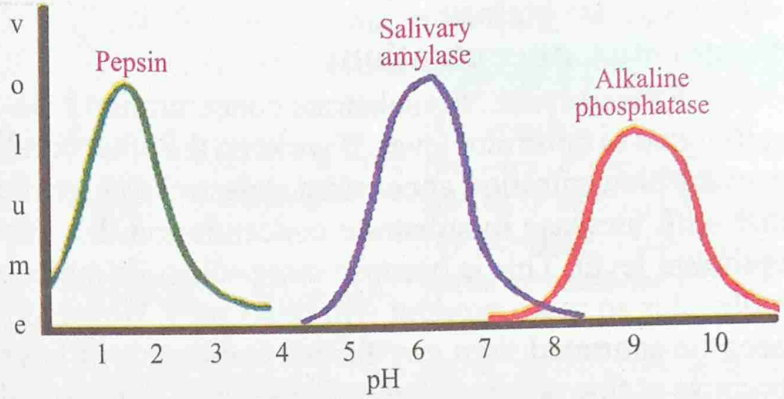


Fig.3.6 Effect of pH on enzyme action

Activity

Find the pH of different food substances by searching internet.

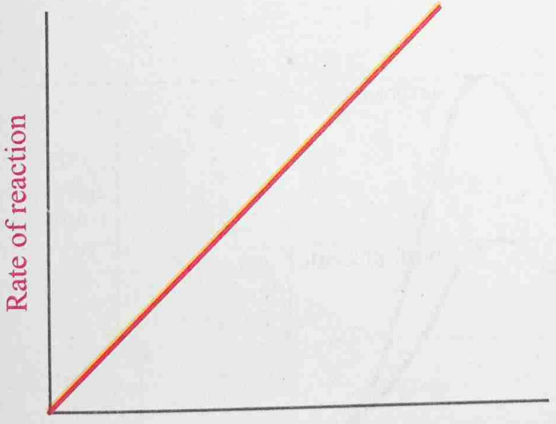


Fig. 3.7 Enzyme Concentration

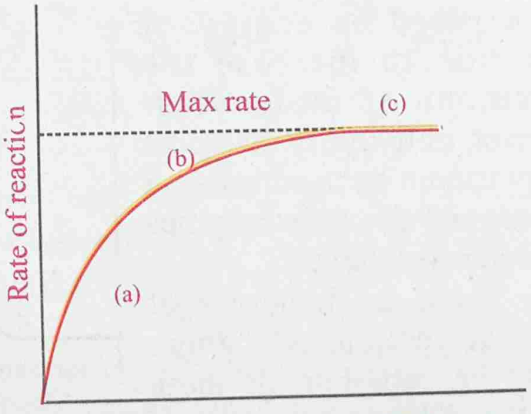


Fig. 3.8 Concentration of Substrate

Tit bits

A restriction enzyme is an enzyme that cleaves DNA into fragments. These enzymes are found in bacteria and provide a defense mechanism against invading viruses. They restrict the entry of foreign DNA in to the host.

Enzyme Concentration:

Enzyme concentration is directly proportional to enzyme activity. If substrate concentration is maintained at high level , and other conditions such as pH and temperature is kept constant then with the increase of enzyme concentration the activity of enzyme will also increase and with the decrease of enzyme concentration the activity of enzyme will also decrease.

Usually in natural conditions the substrate concentration is always high than enzymes. However, when the enzyme concentration become saturated as compared to substrate, then the rate of reaction will not increase further, this maximum rate (Vmax value) is never obtained.

Substrate Concentration:

Like enzymes the substrate concentration is also directly proportional to enzyme activity up to optimum level. If we keep the other conditions such as temperature, pH and enzyme concentration at constant state and change the amount of substrate then we find that with increase in substrate concentration the reaction rate will increase only up to optimum level. This is because more substrate molecules will be colliding with enzyme molecules so more product will be formed. However, at certain concentration substrate become saturated then any further increase will have no effect on the rate of reaction because at this point all the active sites of enzyme will be occupied, maximum rate (V-max).

Energy of Activation(EA):

The minimal amount of energy required to start a chemical reaction is called activation energy. It is denoted by EA and measured in units of kilo joules per mole (KJ/Mol) or kilocalories per mole (Kcal/Mol).

In non-living system, heat is used as energy of activation to increase the movement of molecules. However, in living system heat energy cannot be used because this heat may denature enzymes and proteins of the cell.

There are hundreds of reactions continuously going on in the cell. For all these reactions large amount of activation energy is required. Such a huge amount of energy is not present in living organisms. However, living organisms possess enzymes which lower the activation energy. In the

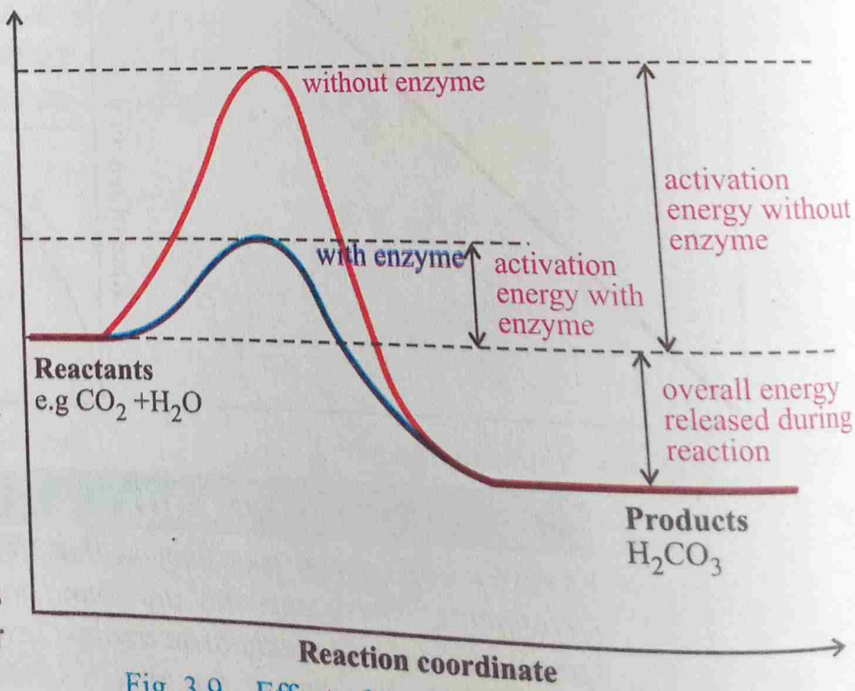


Fig. 3.9 Effect of enzyme on activation energy

presence of enzymes less activation energy is required but in the absence of enzymes more activation energy is required to convert a substrate into product.

3.4 Enzyme Inhibition

The term enzyme inhibition means to stop enzyme from its expression (functioning), usually by enzyme inhibitors or due to change in temperature or pH. Such molecules or substances which stop enzyme activity are called enzyme inhibitors, such as drugs, toxins, products of enzymes etc. Some of the poisons are enzyme inhibitors, that`s why a person exposed to poison may die. On the other hand, there are enzyme activators which bind to enzyme to increase enzyme activity.

Types of Inhibitors:

Generally there are two main types of enzyme inhibitors that is irreversible and reversible inhibitors.

Irreversible Inhibitors:

These inhibitors stop enzyme activity permanently either by destroying (denaturing) the active site of enzyme or occupying active site by making covalent bond with active site. The irreversible inhibitors often contain reactive functional groups e.g., aldehydes, alkenes. These electrophilic groups make covalent bonds with amino acid side chains.

Tit bits

Cyanides are powerful poisons of organisms because they can kill them by inhibiting cytochrome oxidase essential for respiration.

Scientific Knowledge

The enzymes which catalyze chemical reaction again and again are called regulatory enzymes.

The irreversible inhibitors may be natural or artificial e.g., poisons, venom of snakes, drugs etc.

Reversible Inhibitors:

Such inhibitors which attach to enzymes with non-covalent interactions such as hydrogen bond, hydrophobic interactions and ionic bond. These inhibitors generally do not undergo chemical reactions when bonded to enzyme and easily removed from enzymes.

Reversible inhibitors are of two types.

Competitive Inhibitors:

Such inhibitors which have similar shape to the substrate molecule hence compete with substrate to occupy active site. The

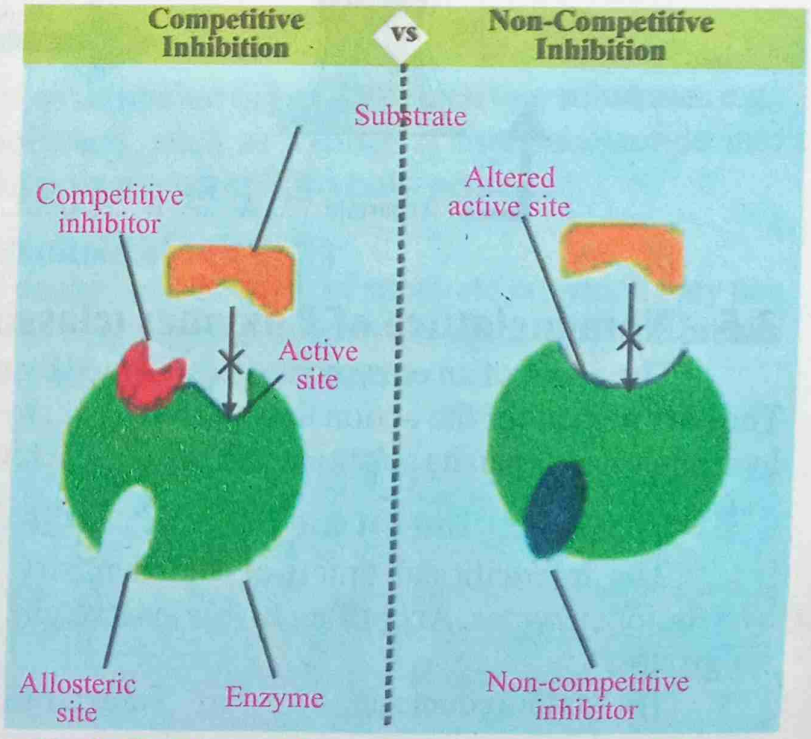


Fig. 3.10 Competitive and Non-competitive inhibitors

process of inhibition depends on the concentration of substrate and inhibitors. With high concentration of inhibitors the chances of inhibition are also high.

Non Competitive Inhibitors:

These inhibitors do not possess structural similarities with the substrate molecule, therefore, attach to allosteric site of enzyme than active site. The attachment of inhibitors changes the shape of active site. Thus substrate cannot bind with active site. Such type of inhibitors are not affected by substrate concentration.

Feed Back Inhibitons:

The production of enzymes, hormones and other products should be in limits to maintain homeostatic conditions. The over production of any product in the body, may prove fatal.

The mechanism through which the production of different products controlled in the body is known as feedback mechanism.

Many enzyme catalyzed reactions are carried out through the biochemical pathways. In these pathways the product of first reaction becomes the substrate for the next reaction. At the end of the pathway a desired product is synthesized. In order to regulate the concentration of that product the biochemical pathway needs to be shut down. This is done through feedback mechanism (automatic system) e.g., the amino acid aspartate changes into threonine through a sequence of five enzymatic reactions. When threonine production become sufficient, it starts accumulating on the allosteric site of enzyme. Thus changes the shape of active site as a result threonine production stops.

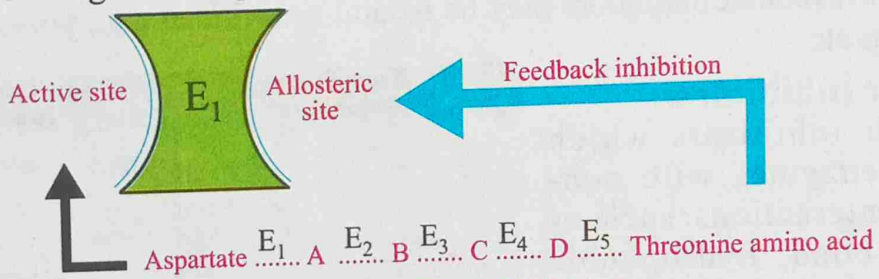


Fig. 3.11 Feedback Inhibition

3.5 Nomenclature of Enzymes (classification of enzymes)

The name of an enzyme is often formed by adding "ase" to the name of substrate. They are named for the action they perform e.g., hydrogenase is an enzyme that removes hydrogen atom from its substrate and cellulase which breaks down cellulose.

1. Classification on the basis of reaction types or functions

The international union of Biochemistry in 1961 has given a nomenclature system for enzymes. According to this system the enzymes are classified into following six groups.

i) Oxidoreductases ii) Transferases iii) Hydrolases
iv) Lyases v) Isomerases vi) Ligases

Oxidoreductases:

These enzymes catalyse different types of oxidation-reduction reactions i.e. removing or adding electrons or hydrogen ions from or to the substrate. The sub classes of these enzymes are oxidases, oxygenases and peroxidases.

Transferases:

These enzymes cause transfer of group from one molecule to another molecule called transferases. Examples of such groups are amino group, carboxyl group, methyl and carbonyl group. Example of transferases enzymes are hexokinases which transfer phosphate group from ATP to glucose.

Hydrolases:

These enzymes break down proteins, fats and carbohydrates by adding water so are called hydrolases e.g., lipase, sucrase, maltase, cellulase, proteinase etc.

Lyases:

These enzymes catalyse the breakdown of specific covalent bond and removal of functional group without hydrolysis e.g., decarboxylase, add or remove carboxyl group, deaminases, add or remove amino group etc.

Isomerases:

Isomers are molecules having similar molecular formula but different structural formula e.g., glucose, fructose and galactose are isomers having same molecular formula $C_6H_{12}O_6$ but have different structures. Isomerase enzymes bring about intramolecular rearrangement within a molecule e.g., phospho-hexose isomerase change glucose 6-phosphate to fructose 6-phosphate.

Ligases:

These enzymes are responsible for formation of bond between two substrates e.g., polymerase joins monomers into polymers, such as joining of mononucleotide into dinucleotide or polynucleotide by DNA polymerase or RNA polymerase.

2. Classification on the basis of name of substrate

Enzymes can also be classified on the basis of name of substrate on which they use e.g., protease breaks protein into amino acids, lipase hydrolyses lipid, amylase breaks down amylose, nuclease acts on nucleic acid, diastase acts on starch etc.

Table 3.2 Comparison between reversible and irreversible enzyme inhibition

Reversible inhibitor	Irreversible inhibitor
1- Binds via non covalent interactions. 2- Do not perform any chemical changes. 3- Can be reversed, as there is no bonding between the inhibitor and substrate.	1- Binds via covalent interactions. 2- Inhibitor binds to the substrate and prevents catalytic activity of enzymes. 3- Irreversibility is due to strong covalent bonding.

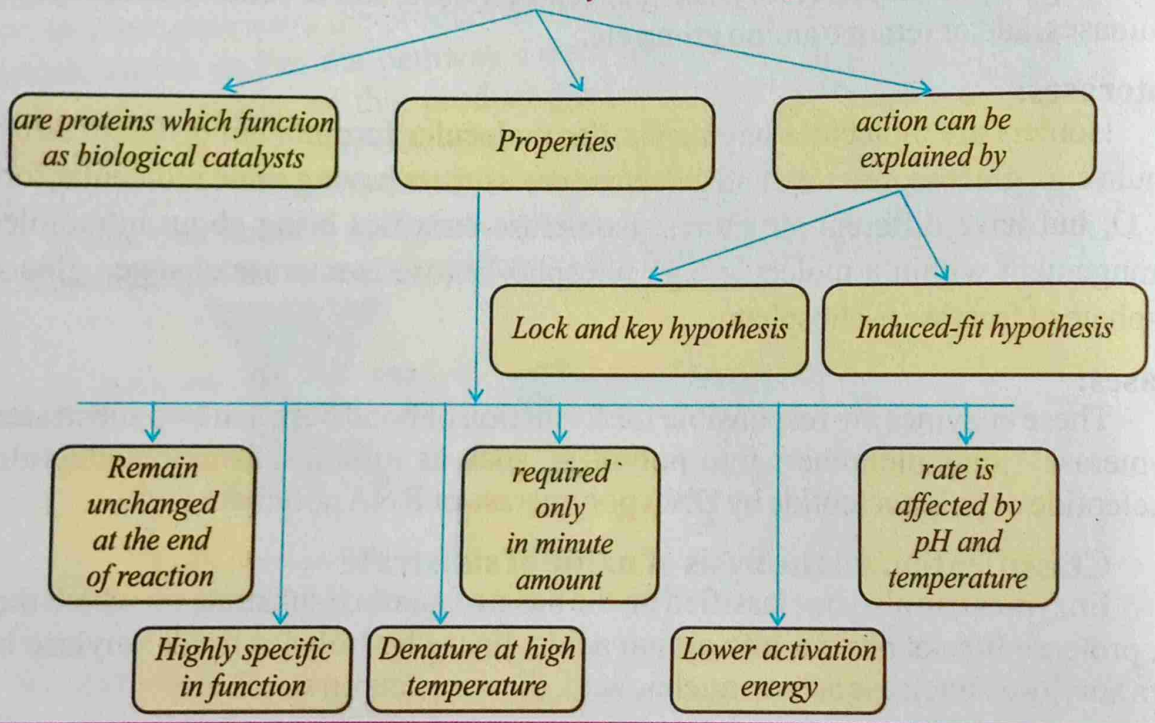
Table: 3.3 Comparison between Competitive and non-competitive enzyme inhibition

Competitive inhibition	Non-competitive inhibition
1- Example: succinate dehydrogenase is inhibited by malonate. 2- Inhibitor binds to active sites. 3- Inhibitor does not change the shape of the active site. 4- Increase in substrate concentration reduces the effect.	1- Example: pyruvate kinase is inhibited by alanine. 2- Inhibitor binds away from the active site i.e., at allosteric site. 3- Inhibitor changes the shape of the active site. 4- Increase in substrate concentration does not affect.

Do you know?

Germinating seeds have enzymes which convert insoluble stored food into simpler soluble substances for example the enzyme amylase digests starch and converts it into maltose.

Table 3.4 A Bird's eye view of Enzymes



Skills: Analyzing

Relate enzyme activity with antibiotics by searching internet and try to find out the reason why antibiotics are not effective against viruses.

Do you know?

Papain enzyme also known as papaya proteinase -1. It is a cystein protease present in papaya and mountain papaya. Active both in acidic and basic medium.

Industrial Enzymes

The commercial use of enzymes is increasing day by day due to the advancements of biological knowledge of enzymes .

The enzymes are used in variety of industries such a pharmaceuticals, chemical productions, Bio fuels, food and beverages industry and consumer products like Laundary detergents, products of cosmetics, meat tenderizers etc.

SUMMARY

EXERCISE

Section I: Objective Questions

Multiple Choice Questions

A. Choose the best correct answer.

1. A biochemical reaction would proceed at a very slow speed making life impossible in the absence of
   (a) Enzyme (b) Cofactor
   (c) Coenzyme (d) Substrate
2. An enzyme with its coenzyme or prosthetic group is called as
   (a) Holoenzyme (b) Apoenzyme
   (c) Activator (d) Inhibitor
3. Generally a single enzyme catalyzes only a single substrate or a group of related substrates, therefore, the enzymes are
   (a) Specific (b) Reactive
   (c) Activator (d) Inhibitor
4. The enzymes involved in cellular respiration are found in
   (a) Golgi bodies (b) Mitochondria
   (c) Chloroplast (d) Ribosomes
5. Every enzyme functions most effectively over a narrow range of pH known as
   (a) Maximum (b) Minimum
   (c) Optimum (d) Both a and b
6. Enzymes are sensitive to minor changes in
   (a) pH (b) Substrate concentration
   (c) Temperature (d) All of these
7. The chemical substance with which an enzyme reacts is called its
   (a) Substrate (b) Active site
   (c) Inhibitor (d) Cofactor
8. Enzymes require which medium for its activity.
   (a) Solid (b) Semi-solid
   (c) Aqueous (d) Jelly-like

9. The optimum temperature for enzymes in human body is
   (a) 4°C (b) 37°C
   (c) 41°C (d) 50°C
10. The catalytic activity of an enzyme is restricted to its small portion called
   (a) Active site (b) Passive site
   (c) Intermediate (d) Allosteric site
11. The reversible inhibitors usually constitute
   (a) Strong linkage with enzyme (b) Weak linkage with enzyme
   (c) No linkage with enzyme (d) Medium linkage with enzyme

B. Fill in the blanks.

1. The detachable cofactor of enzyme is called ..... .
2. Reversible inhibitors may be competitive or ..... .
3. The minimal amount of energy required to carry out a chemical reaction is called ..... .
4. Enzymes become denatured due to ..... temperature .
5. The optimum pH of pepsin is ..... .
6. Induced fit hypothesis was proposed by ..... in 1958.

Section II: Shorts Questions